Ann. N.Y. Acad. Sci. ISSN 0077-8923

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Issue: *The Year in Diabetes and Obesity*

What's the matter with MAT? Marrow adipose tissue, metabolism, and skeletal health

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Marrow adipose tissue (MAT) is functionally distinct from both white and brown adipose tissue and can contribute to systemic and skeletal metabolism. MAT formation is a spatially and temporally defined developmental event, suggesting that MAT is an organ that serves important functions and, like other organs, can undergo pathologic change. The well-documented inverse relationship between MAT and bone mineral density has been interpreted to mean that MAT removal is a possible therapeutic target for osteoporosis. However, the bone and metabolic phenotypes of patients with lipodystrophy argues that retention of MAT may actually be beneficial in some circumstances. Furthermore, MAT may exist in two forms, regulated and constitutive, with divergent responses to hematopoietic and nutritional demands. In this review, we discuss the role of MAT in lipodystrophy, bone loss, and metabolism, and highlight our current understanding of this unique adipose tissue depot.

Keywords: lipodystrophy; marrow fat; osteoporosis; diabetes; marrow adipose tissue

Introduction

Advances in the study of marrow adipose tissue (MAT) have occurred in distinct waves over the past 150 years. In the second half of the 19th century, it was determined that the skeleton is filled with areas containing yellow or red marrow that are distributed in a defined pattern.[1,2] Perhaps owing to the publication of these results in French and German and the difficulty of dissemination of research findings at the time, the idea of there being a distinct pattern of marrow was largely ignored and forgotten until Piney's article "The Anatomy of the Bone Marrow" in 1922.[2] This may have sparked some interest in the topic, and in the 1930s the histological nature and distribution of bone marrow fat was defined, in addition to its possible temperature-dependent regulation in rats.[3–5] Interest waned in the 1940s, though it was proposed that the bone marrow be recognized as a site of conventional fat storage.[6] In the 1950s and 1960s, MAT became fully recognized as fat with the potential for adipose tissue-like character, and the term *yellow marrow* began to fall out of use in favor of the terms *marrow fat* or *fatty marrow* (Fig. 1).[7] The research on marrow fat began to accelerate at this time, ushering in a golden age of MAT research in the 1970s that began with a paper published in *Science* by Mehdi Tavassoli in which intrinsic differences between red and yellow marrow histogenesis were identified.[8] He went so far as to suggest that red and yellow marrow have programmed epigenetic differences, a concept that is of significant interest today, 43 years later.[8] In 1976, Tavassoli, through elegant ultrastructural studies on developing tissue, revealed that the MAT adipocyte develops from a unique progenitor when compared to the white adipose tissue (WAT) adipocyte.[9] This, again, is a line of investigation that is currently of great interest and under active investigation by Mark Horowitz at Yale University, among others. Finally, Tavassoli showed that there are two unique populations of MAT within the bone marrow and that these cells respond differently to hematopoietic demands.[10] We have recently observed this phenomenon in a different model, referring to MAT as either regulated or constitutive,

doi: 10.1111/nyas.12327

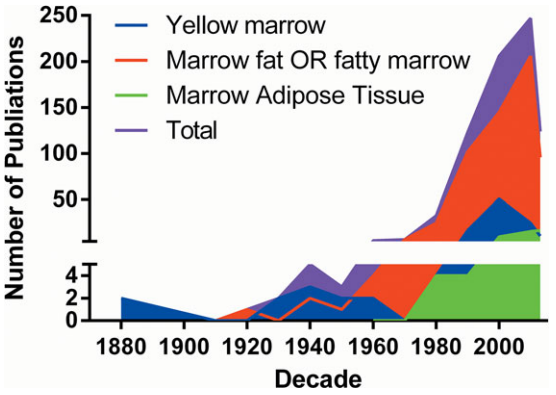

Figure 1. Publications containing the search terms "yellow marrow," "marrow fat/fatty marrow," or "marrow adipose tissue" with time. This graph was generated based on a PubMed search for the terms of interest. Given poor indexing of abstracts before 1950, several additional references were added in manually. In addition to general publication trends, this demonstrates a shift in nomenclature from "yellow marrow" to "marrow fat/fatty marrow" in the 1950s and 1960s. Though there was a resurgence in the use of the term "yellow marrow" in the 1980s, its use is declining again in favor of "marrow adipose tissue."

and are actively working to define its molecular basis. The cells interspersed with red marrow, which we refer to as *regulated MAT* (rMAT), are depleted in response to phenylhydrazine-induced hemolysis, while those in other regions, referred to as *constitutive MAT* (cMAT), are preserved.[10]

In the late 1970s and 1980s the term MAT began to appear in selected manuscripts in recognition that the marrow fat exists as a distinct adipose tissue organ (Fig. 1). After a period of discovery which focused on elucidating the basic nature of MAT, in the 1980s the bone biology community became very concerned about the potential relationship between MAT accumulation and bone loss. This led to many clinical and animal studies on the topic, casting MAT as a "villain" in diseases such as osteoporosis.[11,12] Today, research on the relationship between MAT and skeletal metabolism occurs in many groups on a worldwide scale and publications containing the terms *marrow fat* and *MAT* have increased each decade since the 1970s (Fig. 1). In addition to understanding the impact of MAT on bone, several groups have recently picked up where Tavassoli left off and are working diligently to define the MAT progenitor and to understand the fundamental genetic, epigenetic, and metabolic differences between MAT and WAT. This review will explore what we know about the relationships between MAT, bone, and the body and provide several hypotheses regarding the role of MAT in regulation of both skeletal and endocrine metabolism.

Does the skeleton need fat to be metabolically healthy?

Under the light microscope, MAT looks histologically identical to endocrine and mechanical WAT (Fig. 2). Mechanical WAT is the adipose tissue that functions as physical padding, for example, on the palms and soles of hands and feet. Despite similarities to endocrine WAT, we know that mechanical WAT is relatively metabolically inert, while endocrine WAT plays a major role in satiety, fertility, and glucose homeostasis.[13] Like the differences between WAT types, MAT is also distinct in both its gene expression and ability to respond to nutritional status.[14] The most puzzling example in recent years is that in states of calorie restriction and anorexia, the amount of MAT increases while peripheral WAT is lost.[15,16] In addition to WAT, MAT has been likened to a third type of adipose, the brown adipose tissue (BAT).[17] BAT is functionally and histologically distinct from WAT and converts energy from glucose and triglycerides into heat, an integral aspect of adaptive thermogenesis (Fig. 2).[18] Like MAT, both WAT and BAT have the ability to regulate skeletal metabolism.[19–21]

Largely owing to the use of marrow as an energy substrate, it has long been recognized that bone marrow composition varies by site, with the more red marrow being confined toward the middle of the animal and the yellow, or fatty, marrow existing on the periphery (Fig. 3A).[2] The earliest fatty change of the human marrow in the phalanges of the toe begins at or slightly before birth, regardless of prematurity, and rapidly accelerates between 4 and 8 weeks of age (Fig. 3B).[22] The marrow in the phalanges gradually matures and reaches full fatty conversion after 1 year (Fig. 3B).[22] MAT continues to accumulate in the appendicular skeleton from distal to proximal until age 20–25, with gradual MAT conversion continuing in the axial skeleton throughout life (Fig. 3C).[2,23] A similar, highly regulated pattern of development is recapitulated in many vertebrate species including mice, rats, and rabbits.[24,25] In rabbits, for example, the mature pattern of MAT conversion occurs by age 4–6 months. At birth, sites of high MAT like the rabbit tibia have a relatively low density of hematopoietic elements when compared to sites of

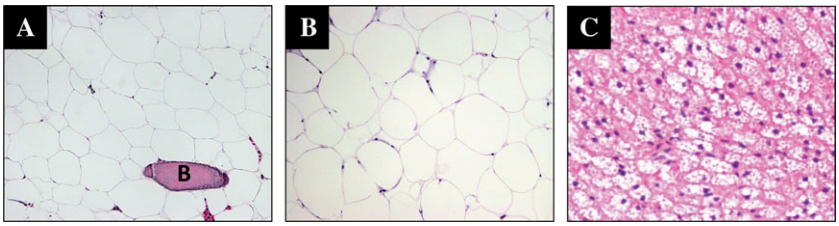

Figure 2. Histology of MAT, WAT, and BAT. (A) Human marrow adipose tissue, B: bone. (B) Human subcutaneous white adipose tissue from the thigh. (C) Mouse brown adipose tissue. H&E stain, 200× magnification.

low MAT such as the lumbar vertebrae.[25] As MAT develops, its density is inversely proportional to this initial cellularity, suggesting that the distribution of MAT is programmed from birth.[25]

Genetic insights into the MAT–skeletal relationship

Lipodystrophy in humans

Lipodystrophy is a complete or partial loss of body fat. Surprisingly, loss of body fat can result in insulin resistance and diabetes. This is generally accompanied by pathologic accumulation of lipid in the liver and other sites, suggesting that a normal amount of endocrine adipose tissue is necessary for proper glucose homeostasis.[26] Patients with lipodystrophy have low circulating levels of the adipokines leptin and adiponectin.[26] Lipodystrophy is classified as generalized or partial depending on the extent of body-fat loss, and has been linked to genetic mutations in genes including *AGPAT2*, *BSCL2*, *CAV1*, and *PTRF* (Table 1, Fig. 2).[26] Depending on the genetic variant, lipodystrophy occurs as either a failure of adipocyte formation from birth or normal formation of adipocytes with a failure of adipocyte maintenance and gradual loss of WAT mass over time. The specific genetic mutations have different effects on endocrine WAT, mechanical WAT, and MAT (Table 1, Fig. 4). The bone phenotype also varies between subtypes. Comparisons between lipodystrophic phenotypes can provide insight into (1) the impact of MAT on the skeleton and (2) the genetic and functional differences that exist between WAT and MAT.

In patients with *AGPAT2*-associated type 1 congenital generalized lipodystrophy (CGL1), both endocrine WAT and MAT are lost while mechanical WAT is maintained (Table 1, Fig. 4).[28,29] In contrast, *BSCL2*-linked lipodystrophy (CGL2) results in loss of all forms of adipose tissue.[28] *AGPAT2* encodes for 1-acylglycerol-3-phosphate-*O*-acyltransferase 2, which catalyzes the formation of phosphatidic acid, critical for triacylglycerol synthesis in adipose tissue, suggesting that both MAT and endocrine WAT rely on this enzyme for synthesis and subsequent storage of triacylglycerol. The *BSCL2* gene encodes for seipin, a regulator of adipocyte differentiation and lipid droplet formation.[30] Loss of all fat with *BSCL2* mutation implies that seipin functions upstream of adipocyte formation and/or lipid storage in all three fat types. CGL3 and CGL4 are associated with mutations in *CAV1* and *PTRF*, respectively.[31,32] *CAV1* encodes caveolin 1, a key structural component of 50- to 100-nm invaginations of the plasma membrane called caveolae.[33] Caveolae account for 20–40% of the surface of the adipocyte and are major regulators of insulin signaling and lipid trafficking.[26] *PTRF* encodes for cavin, a protein required for stabilization of caveolins and formation of caveolae.[31] Unlike CGL1 and CGL2, MAT is retained in both CGL3 and CGL4, suggesting that MAT can form and store lipids in the absence of caveolae.[31,32]

CGL was initially reported in 1954 by Berardinelli, with additional reports in greater detail by Seip in 1959 leading to its designation as Berardinelli–Seip Syndrome, a name now synonymous with CGL1 or CGL2.[34–36] Mutations in the *AGPAT2* or *BSCL2* genes account for ∼95% of cases of CGL.[37] Longitudinal studies in which patients with CGL were followed for up to 38 years, in addition to standard case reports, have allowed for a fairly precise characterization of the bone phenotype.[34,38–40] Ninety-three patients with CGL in which some form of skeletal analysis was performed are detailed in Table S1 and summarized in Table 1.[28,31,32,34–36,38–56]

Our understanding of the skeletal differences between CGL subtypes is somewhat limited since the CGL-causing genetic mutation is unknown in the

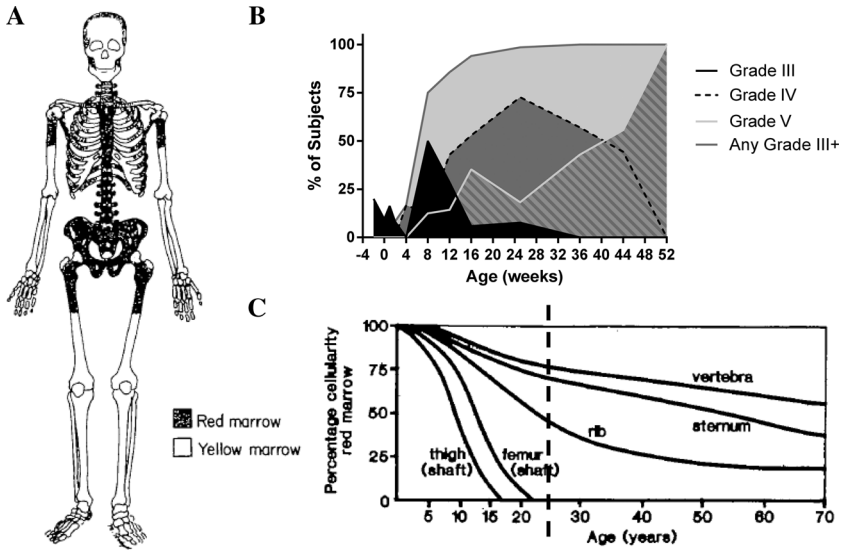

Figure 3. Distribution of marrow adipose tissue and changes skeletal adiposity with time. (A) Distribution of marrow adipose tissue (yellow marrow) and hematopoietic or red marrow in an adult skeleton.[23] (B) The percentage of subjects with fatty conversion of the marrow in the phalanges of the 2nd toe at autopsy relative to birth (age 0 weeks). Grade III: some mature adipocytes present, Grade IV: mostly fat with some islands of hematopoiesis, Grade V: complete fatty conversion.[22] (C) Loss of red marrow in the appendicular (femur) and axial skeleton (rib, sternum, and vertebra) with age. Dashed line: approximate age of mature MAT phenotype in the appendicular skeleton.[23]

majority of early reports. However, in 2004, Fu *et al.* identified the genetic mutation in 26 families with CGL and concluded that the phenotypic characteristics of patients with *AGPAT2* (CGL1) and *BSCL2* (CGL2) mutations are similar, with the exception that mental retardation is more prevalent with *BSCL2* mutation (Table 1).[55] Since ∼95% of patients with CGL have been found to harbor mutations in either *AGPAT2* or *BSCL2*, if we group all CGL cases of unknown genetic origin with those identified as CGL1 and CGL2, identifiable patterns in skeletal development begin to emerge (Table 1).[37] In the first decade of life, patients with CGL1 or CGL2 present with prominent muscularity, accelerated bone growth, advanced skeletal age (>3 years above their chronological age), cortical thickening, and skeletal sclerosis.[38,41,48,53,55,56] Children with CGL1 or CGL2 are initially tall, and up to 90% of their growth occurs in the first 10 years of life.[44] After this period, growth slows, and adults with CGL are of average stature.[44,57] As the children age, there is often an increase in radiodensity in the appendicular skeleton, accompanied by metaphyseal sclerosis with similar, though less prevalent, changes noted in the axial skeleton.[42,53] The mechanism is unknown, but this may represent a shift in osteoblast activity or number in response to the absence of MAT that would normally be forming in the appendicular skeleton at this age (Fig. 3C). It could also be a response to the muscular hypertrophy associated with CGL or potentiation of osteoblast activity by excess circulating insulin.[58,59] The skeletal development of patients with CGL3 (*CAV1*) or CGL4 (*PTRF*) raises doubts about the latter possibilities.[31,32,50,51] Like those with CGL1 and CGL2, patients with CGL4 tend to have accelerated skeletal growth during the first year of life, however, this rapidly slows and skeletal age is normal or decreased in childhood. In addition, sclerosis is not a common feature of CGL3 or CGL4, rather, bone density tends to decrease, in some cases leading to development of osteopenia or osteoporosis. This may be linked to the persistence of MAT in patients with CGL3 and CGL4 that is not normally present in CGL1 or CGL2 (Table 1, Fig. 4).

In adolescence, a subset of patients with CGL1 or CGL2 develop osteolytic cyst-like lesions in the long bones and occasionally the phalanges that are progressive and can result in pathologic fractures (Fig. 5).[38,41,43,47,49,55] Biopsy results have been reported in several cases and generally contain bone fragments, normal hematopoietic marrow, and a proliferation of vascular structures that has been

Table 1. The adipose tissue phenotype, skeletal changes, and clinical findings associated with CGL1, CGL2, CGL3, and CGL4

Adipose tissue phenotype	CGL1: AGPAT2	CGL2: BSCL2	CGL3: CAV1	CGL4: PTRF
Visceral WAT	Absent	Absent	Absent	Absent
Subcutaneous WAT	Absent	Absent	Absent	Absent
Mechanical WAT	Present	Absent	Partial loss	Partial loss
MAT	Absent	Absent	Present	Present
Skeletal phenotype	CGL1/2/Unknown (75 Total)		CGL3/4 (18 Total)	
Age 0–1				
Rapid growth/advanced skeletal age	2/2 (73 ND)		ND	10/10 (8 ND)
Sclerosis	3/4 (71 ND)		ND	
Bone density	Increased 4/4 (71 ND)		ND	
Age 1–10				
Advanced skeletal age	23/25 (50 ND)		ND	0/12 (6 ND)
Sclerosis	10/15 (60 ND)		ND	0/10 (8 ND)
Bone density	Increased 7/7 (68 ND)		ND	
Bone cysts	0/13 (62 ND)		ND	
Age 10–20+				
Advanced skeletal age	1/3 (72 ND)		ND	1/3 (15 ND)
Sclerosis	9/10 (65 ND)		0/1	ND
Bone density	Increased 10/10 (65 ND)		Low	Low 2/2 (16 ND)
Bone cysts	22/32 (43 ND)		0/1	0/1 (17 ND)
Clinical findings	CGL1/2/Unknown (75 Total)		CGL3/4 (18 Total)	
Acanthosis nigricans	55/62 (13 ND)		1/1	0/18
Prominent musculature	52/52 (23 ND)		1/1	17/18
Diabetes	33/48 (27 ND)		1/1	0/18
Age of diabetes onset	12.1 ± 1.2 Years Old (N = 12)		Age 13	N/A
High serum triglycerides	46/57 (18 ND)		1/1	4/16 (2 ND)
High serum insulin	16/19 (56 ND)		1/1	4/16 (2 ND)
Mental retardation	0/10 (4 ND)	3/5 (7 ND)	0/1	0/7 (10 ND)

Note: This table summarizes the findings from Table S1, which lists data from 93 patients with congenital generalized lipodystrophy (CGL) in which some form of skeletal analysis was performed. Data was not reported for all parameters on each patient and they are indicated as ND, no data. CGL. congenital generalized lipodystrophy, AGPAT2. 1-acylglycerol-3-phosphate-*O*-acyltransferase 2. BSCL2. Berardinelli-Seip congenital lipodystrophy 2. CAV1, caveolin 1. PTRF, polymerase I and transcript release factor. WAT, white adipose tissue. MAT, marrow adipose tissue.

likened to cystic angiomatosis.[41,49] In one case, an epithelial cyst lining was also noted.[47] The factors leading to cyst formation have sometimes been attributed to environment, as in three cases not all CGL-affected members of the same family demonstrated evidence of cystic bone changes (Table S1).[38,47,55] However, in two of the three reports, the family members without osseous cysts were age 11 or younger.[47,55] Thus, the unaffected family members may have simply been too young for the cystic phenotype to be present (Table 1). Given the lack of marrow fat in these patients, the expected development of MAT and conversion of red to yellow marrow during childhood and adolescence fails to occur (Fig. 3). Thus, cyst formation at this time may represent a local reaction to the failure of MAT conversion. This is further supported by skeletal findings in acquired generalized lipodystrophy (AGL), CGL3, and CGL4. In AGL, the adipose tissue forms normally but WAT is subsequently lost during childhood and adolescence.[60] In contrast to *AGPAT2*- and *BSCL2*-linked CGL, MAT in patients with AGL, CGL3, and CGL4 is generally preserved (Fig. 4).[60] Perhaps due to the preservation of MAT in AGL, cystic bone lesions of any type are exceedingly rare and have only been noted in four of ∼64 cases in the literature (Fig. 5).[58,61] Similarly, they have not been documented in any patients with CGL3 or CGL4. This is in contrast to the presence of cysts in 22 of 32 patients with CGL1, CGL2, or CGL of

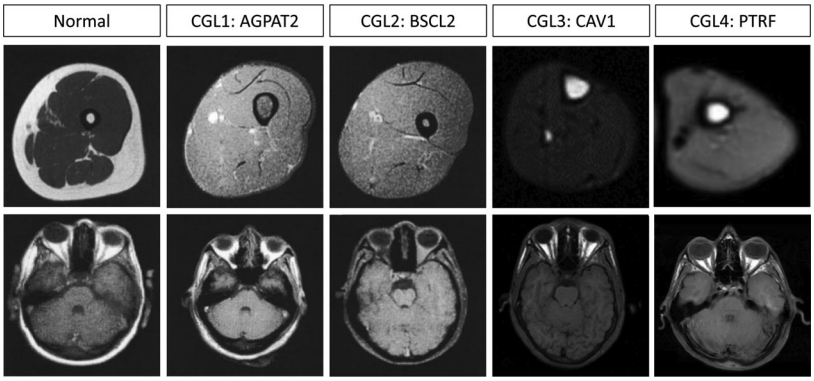

Figure 4. **T1-weighted MRI images of WAT and MAT in CGL. Transverse T1-weighted MRI cross-sections of the limb (top row) and skull at the level of the orbits (bottom row) in patients with CGL as compared to control. In the limb of a normal control a thick layer of subcutaneous adipose tissue (white) is present around a core of muscle (gray). In the center is bone (black) that is filled with marrow adipose tissue (white). Both subcutaneous and marrow adipose tissue are absent in CGL1 and CGL2. In CGL3 and CGL4, subcutaneous fat is lost while marrow fat is maintained. In the cranial section, a layer of mechanical white adipose tissue is present around the skull and behind the orbits. Both mechanical depots are maintained in CGL1, both are lost in CGL2, and the fat behind the orbits but not around the skull is present in CGL3 and CGL4. Images for normal (age 30, female, femur), CGL1 (age 31, female, femur), and CGL2 (age 11, female, femur) from Ref. 28; CGL3 (age 19, female, tibia) from Ref. 32; and CGL4 (Age 14, male, humerus) from Ref. 50.**

unknown type that were monitored to or past adolescence and checked for cyst formation (Table 1, Table S1). These findings support the notion that MAT plays an important role in the maintenance of skeletal homeostasis.

There are several important observations that can be derived from the human condition. First, neither MAT nor WAT is necessary for basic skeletal patterning and formation. However, loss of WAT in CGL is sufficient to induce skeletal changes ranging from osteosclerosis, advanced bone age, and cortical thickening (in the absence of MAT) to osteopenia and osteoporosis (when MAT is maintained). Second, retention of MAT may protect the skeleton from cyst formation during adolescence and pathologic fractures later in life. These findings suggest a bell-shaped curve in which both too little MAT or too much MAT are not ideal. In addition to effects on the skeleton, the lipodystrophies raise the possibility that MAT may be able to functionally contribute to systemic metabolism—especially in times when WAT is decreased or absent. It is clear that the presence of MAT is not sufficient to completely compensate for the loss of endocrine WAT, however, the prevalence of hyperinsulinemia, high serum triglycerides, acanthosis nigricans (a sign of insulin resistance), and diabetes is lower in patients with CGL4 when compared to CGL1 or CGL2 (Table 1). This could imply that preservation of MAT in CGL decreases the severity of diabetes and insulin resistance. The rarity of reports limits any definitive conclusions but suggests that further investigation of this relationship may prove fruitful. For example, preservation of MAT may improve insulin sensitivity through secretion of adipokines, including adiponectin, into the systemic circulation. In mice, adiponectin decreases insulin resistance by limiting lipid storage in muscle and the liver; furthermore, restoration of circulating adiponectin can help to reverse the insulin resistance associated with lipodystrophy.[27,62]

Genetically engineered mouse models

CGL is exceedingly rare, estimated to affect only one in every 5–10 million people in the United States.[60] Thus, mouse models with similar genetic mutations have been generated to provide phenotypic and mechanistic insights. Mice with CGL tend to have phenotypes that are significantly less severe than humans. CGL1 *AGPAT2*$^{-/-}$ mice most closely mimic the human condition with nearly absent adipose tissue, diabetes, high circulating insulin and triglycerides, and low circulating leptin.[63] Unfortunately, information regarding the bone phenotype of these animals has not been published. Three different *BSCL2* knock-out mice have been made, all of which show a 50–70% reduction in WAT and BAT, diabetes, high circulating insulin,

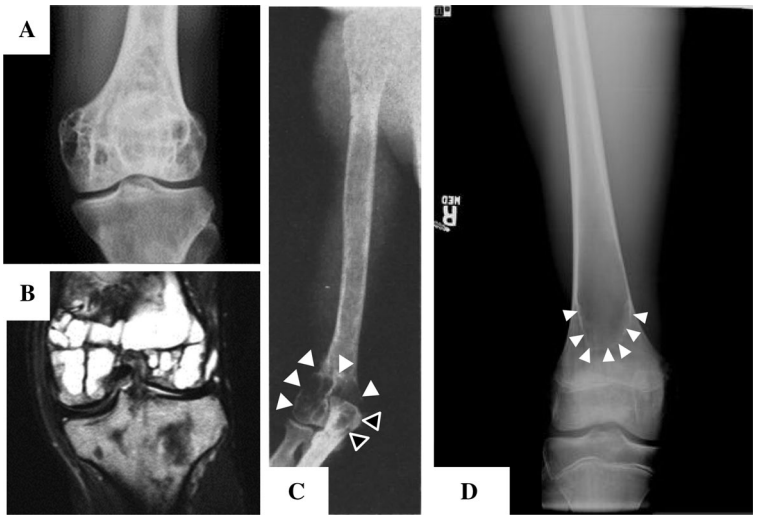

Figure 5. **Osteolytic skeletal lesions in CGL and AGL. (A), (B) Multicystic transformation of the distal femur in a 42-year-old Kurdish woman with CGL.[54] The lesion remained relatively unchanged between age 31 and 42 and showed coarse trabeculation of cystic areas filled with high, fluid-like signal intensity on T2-weighted MRI images.[54] (C) Bilateral lesions of the distal humeri, distal radii, proximal and distal ulnae, and phalanges of the hand were noted in a 17-year-old female with CGL that became progressively worse by age 22 and resulted in pathologic fracture of the right humerus at age 23 and the left forearm at age 24.[47] Lesions of the left distal humerus (white arrows) and proximal ulna (black arrows) are shown in panel C. (D) Bilateral unicameral bone cysts of the femoral diaphysis (right femur pictured) in a 13-year-old male with acquired general lipodystrophy.[61] Distal extension noted by the white arrows.**

and low circulating leptin.[64–66] However, the normal baseline increase in circulating triglycerides is not present and again, bone phenotyping has not yet been published.[64–66] *CAV1* (CGL3) and *PTRF* (CGL4) knock-out mice have also been generated and reveal an even wider divergence from the striking human phenotype. Instead of near complete loss of body fat, WAT is reduced by only up to 40%. Specifically, *CAV1*$^{-/-}$ mice appear quite similar initially and reveal their adipose tissue phenotype only with age or dietary manipulation. This phenotype consists of small adipocytes and a robust resistance to high-fat diet–induced obesity.[67] Unlike the human report of a *CAV1* mutation, these mice do not have diabetes or high circulating insulin, though high serum triglycerides and low levels of adipokines persist.[67] The bone volume of *CAV1*$^{-/-}$ mice is significantly increased at 5–8 weeks of age due to an underlying increase in the bone formation rate.[68] The bone phenotype of *PTRF* knock-out mice is currently unknown.[69]

Adipokines

Adipocyte-secreted cytokines (*adipokines*) such as leptin can regulate bone formation through central induction of the sympathetic nervous system.[70,71] Clinical studies including the 24 reports detailed in Table S2 have revealed, if anything, a mild, gender-specific correlation between serum leptin level and bone mineral density in humans (Table S2). While leptin often correlates positively with BMD in females,[72–90] the opposite is noted in males.[72,74,75,81,82,85,86,88,91–94] In the genetic models of lipodystrophy, both leptin and adiponectin circulate at low levels despite highly divergent bone phenotypes.[27] Simultaneous restoration of both leptin and adiponectin, or in some cases leptin alone, is sufficient to normalize insulin sensitivity in lipodystrophic mice.[62,95] Similarly, in humans, recombinant leptin has been used in patients with difficult-to-treat lipodystrophy, resulting in improvements in insulin sensitivity, glucose tolerance, and body composition.[96] Despite significant improvements in metabolic parameters and restoration of physiologic leptin levels in humans, recombinant leptin did not change bone mineral density after 4–18 months of replacement therapy.[96–98] This may be due to the small sample size of the study, inclusion of different forms of lipodystrophies, or the length of follow-up, and does not rule out the possibility that leptin,

or other adipokines, can regulate bone formation—especially when present in excess. However, since regulation of bone by adipokines has been covered extensively in previous reviews, we will not include any further discussion here.[99]

MAT and bone loss

Human and animal models

Since the 1980s, it has been a recurring paradigm that MAT is a negative regulator of bone formation. Human studies have correlated increases in MAT accumulation with decreases in cortical bone,[100] low bone mineral density,[101] decreases in bone volume,[102] decreased bone formation rate,[102] osteoporosis, and osteopenia.[103] Despite these correlations, it is unclear whether increases in MAT directly regulate bone formation and strength or simply represent a passive response to bone loss or changes in the marrow microenvironment. For example, MAT is increased in premenopausal women with idiopathic osteoporosis.[102] However, the expected negative correlations between MAT volume and bone formation, while present in the control population, were not present in those with idiopathic osteoporosis despite loss of bone density and increased rate of fracture.[102] This implies that MAT accumulation is not necessary for loss of bone integrity, at least in some situations. This is further supported in rodent models.[104] For example, mice deficient in 11β-hydroxysteroid dehydrogenase type I fail to develop marrow fat in the proximal tibia, yet maintain the same skeletal architecture and rate of bone loss with age when compared to wild-type mice.[104] Similarly, Tornvig *et al.* induced MAT formation in mice with troglitazone and concluded that in the absence of a challenge where production of blood or bone is necessary, the accumulation of adipose in the bone marrow does not change the trabecular volume.[105]

It is established that the adult C57BL/6J mouse tibia, one of the fattiest long bones of any skeleton, contains <5% MAT by volume based on osmium staining and CT analyses (unpublished observation). This is consistent with the study referenced above, which observed an increase in MAT at the proximal tibia from $0.2 \pm 0.3\%$ to $4.7 \pm 2.1\%$ after treatment with troglitazone.[105] In the C3H/HeJ mouse strain, baseline levels of MAT increase to ~20% of marrow volume (unpublished quantification). This is in dramatic contrast to an adult human tibia which is filled with 70–100% MAT.[23] There are exceptions, but it seems that the larger the model, the fattier the marrow. Rats are closer to the human condition and, on the basis of a review of bone histology, older males tend to contain closer to 30–50% MAT by volume (unpublished observation). Rabbit tibias, one of the most popular models historically for MAT research, reach levels of 70% or more. The proximal femur, normally about 40–50% MAT by volume in humans, is also essentially the same in rabbits.[106] Variations between species make model selection critical for MAT research, since any impact of MAT on skeletal or systemic metabolism in mice is likely to be amplified in larger animals, including humans.

In order to quantify the relationship between MAT formation and bone loss, long-term temporal studies have been performed. Even with this approach, interpretation is challenging in the absence of a negative control model that resists MAT accumulation. For example, in a temporal study by Li *et al.*, osteoporosis was induced in 5-month-old New Zealand White rabbits with 1.5 mg/kg/day methylprednisolone sodium succinate for 4 weeks, followed by 0.35 mg/kg three times per week for an additional 8 weeks.[106] MRI and DXA scans of treatment and control groups were completed at 4, 8, and 12 weeks.[106] Marrow lipids were significantly increased in the proximal femur by week 4 (+35%) at which time bone mineral density (BMD) was decreased on DXA by 4.5%, though this was not statistically significant. The pattern continued to progress at week 8 with a significant 60.5% increase in marrow lipids and a 13.3% decrease in BMD when compared to baseline readings. However, at week 12, despite an additional 11.8% increase in marrow lipids on MRI and a 26% increase in adipocyte volume by histology, BMD remained unchanged. Thus, both BMD and MAT are regulated by glucocorticoid treatment. In addition, changes in MAT occur more rapidly and to a greater extent. However, in the absence of a control population in which MAT formation was blocked, this fails to prove that MAT accumulation directly regulates BMD. A similar study that analyzed temporal changes in MAT and bone volume in rats with OVX-induced osteoporosis further argues this point. At 4 weeks after OVX induction, the authors observed a 47.4% decrease in trabecular bone volume at the tibial metaphysis with no change in the volume of adipocytes.[107] By 12 weeks post-OVX there was, however, a 173%

increase in MAT volume in addition to a 40.6% loss of bone volume relative to control animals.[107] Again, these results suggest that both MAT and bone volume are regulated in response to OVX. However, in this case bone loss occurred before changes in MAT.[107] It may be that MAT precedes bone loss in some forms of osteoporosis (i.e., glucocorticoid-induced) but not others. If this becomes well defined with temporal studies in humans, even in the absence of a causal relationship between MAT and bone loss, MAT accumulation may be able to serve as a biomarker to alert the provider that the patient is at high risk for future loss of BMD.

Histological studies in rats and dogs have demonstrated decreased osteoblast activity, osteoclast numbers, and bone formation rate at sites of high marrow fat when compared to sites of low marrow fat.[11,108] Sites with high levels of MAT also have a diminished response to osteoanabolic agents including basic fibroblast growth factor and intermittent parathyroid hormone in rodents.[109,110] It may be that MAT decreases osteoblast and osteoclast activity, effectively slowing bone turnover and thus decreasing the rate of bone accumulation or loss.[11,108] For example, the presence of MAT actually protects against cancellous bone loss in rats with OVX-induced osteoporosis.[108,111] In addition to decreasing the rate of bone turnover, it is possible that marrow fat crowds out T cells that have been shown to participate in OVX-induced bone loss.[112] Fatty marrow can also aromatize circulating androgens to estrogen, which may decrease the effects of OVX at sites of high MAT.[113,114]

In summary, it seems indisputable that both MAT accumulation and bone loss occur with age and are temporally linked in many conditions. While it is possible that in some cases the marrow adipocytes or their formation contributes to loss of skeletal integrity, there is more likely a context-specific nature to the appearance of marrow fat that may influence bone remodeling in several ways.

Two types of marrow fat

In addition to its divergence from WAT, MAT may exist in two forms. Tavassoli, in 1976, demonstrated the presence of two different types of adipocytes in rabbit marrow—those that stain with performic acid Schiff (PFAS) and those that do not.[10] The PFAS reaction relies on oxidation of the ethylenic linkages in unsaturated fats to aldehyde and processing with Schiff reagent to generate a red/purple color. This suggests that bone marrow adipocytes interspersed with hematopoietic elements may contain a different class of fatty acids than those without adjacent hematopoiesis. These two populations respond differently to induction of hematopoiesis and anemia. As discussed above, the rMAT cells interspersed with red marrow are depleted in response to phenylhydrazine-induced hemolysis while the cMAT is preserved.[10] According on the literature, rMAT and cMAT may also respond differently to starvation. Cohen, in 1965, placed rabbits on a water-only diet for 28 days and observed a 50–75% reduction in total marrow lipid in the ribs, humerus, femur, tibia, radius, and ulna.[115] Though not directly quantified, it was mentioned that the distal bones preserved more of their MAT than the proximal bones. They also mentioned that the marrow had a gelatinous appearance, which may be similar to the serous transformation that occurs in the marrow of severely anorexic patients.[116] Tavassoli went on to demonstrate that the cMAT at the distal tibia is not mobilized in the rabbit even after 10 days of starvation and loss of 22% of total body mass, further confirming its resilience.[117] The concept that rMAT and cMAT may respond differently to nutritional status is interesting, especially given recent publications that show that MAT increases in states of anorexia and calorie restriction.[15,16]

We hypothesize that cMAT is programmed to develop in a very specific temporal and spatial pattern prior to age 25, while rMAT forms more gradually throughout life. In rodent models, cMAT is found in the tail, paws, and distal tibia, while rMAT is present in the proximal tibia and femur. It is also possible that rMAT forms first and matures into cMAT with time. If true, this has implications for diseases including osteoporosis, which has been associated with a decrease in MAT unsaturation.[118] A shift in marrow fat composition to higher levels of saturated lipid has also recently been correlated with fragility fractures in postmenopausal women.[119] Thus, perhaps it is not simply the presence of MAT but rather a specific type of MAT that negatively affects bone density and metabolism. For example, the paratrabecular distribution of marrow fat accumulation in osteoporosis is dramatically different than the centralized pattern of marrow fat in plasmacytoma (Fig. 6).[12]

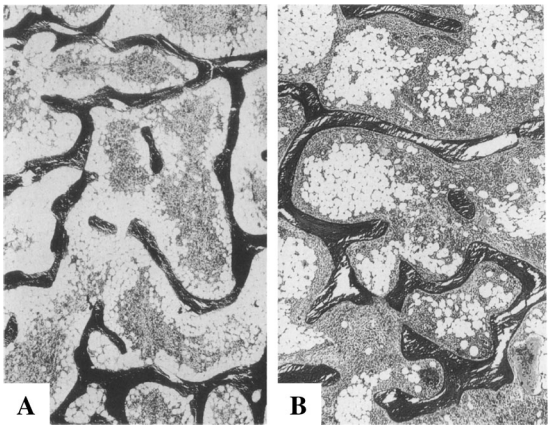

Figure 6. **Distribution of marrow adipose tissue in osteoporosis and plasmacytoma. (A) 75-year-old female with osteoporosis and paratrabecular accumulation of marrow adipocytes. (B) Increase in marrow adipocytes in central spaces in a patient with plasmacytoma.[12]**

MAT and systemic metabolism

Skeletal health and total MAT are acutely responsive to changes in systemic metabolism (reviewed elsewhere).[120] However, the capacity of MAT to actively contribute to glucose homeostasis or energy balance is unclear. As noted in lipodystrophy, patients with *PTRF* mutations have a decreased prevalence of insulin resistance and diabetes when compared to those with *AGPAT2* or *BSCL2* mutations (Table 1). There are many possible explanations, but one consideration is that MAT is maintained in the former but lost in the latter. This prompts the question, Is there enough MAT to actually contribute to systemic metabolism? MRI has been used to quantify total body fat and compare it to the total volume of MAT in hundreds of adult patients (average age 40–50 years).[101,121] The total MAT volume ranges from 0.51 to 3.28 L (mean ~1.5 L). In these patients, MAT made up 1.1–43.2% (mean ~7%) of the total adipose tissue volume.[101,121] The men and women in these studies were of relatively normal size and had an average BMI of 24–28 kg/m^2.[101,121] Similar results were obtained in a younger cohort of 280 men and women (average age ~30), with the proportion of MAT ranging from 1.1% to 31.6% (mean 8.0%) of total adipose mass.[122] These patients had an average BMI of 25–26 kg/m^2.[122] In contrast, the BMI of an average anorexic patient is approximately 17–18 kg/m^2 due to loss of body fat.[16] The proportion of MAT is further amplified in these patients, since MAT, unlike WAT, increases during calorie restriction and is high in anorexic patients.[15,16] Thus, we estimate that the normal proportion of MAT would be robustly amplified both in individuals with anorexia and those with MAT-sparing lipodystrophy, to levels exceeding 30–40%. Based on these findings, it would appear that MAT in humans is of a large enough size and proportion relative to peripheral WAT to make a significant contribution to metabolism, especially in states of low body fat.

So, why does MAT increase in calorie restriction and anorexia? The fundamental mechanism remains under investigation, but several hypotheses regarding the function of MAT have been proposed. For example, MAT may secrete adipokines, such as adiponectin, that can increase insulin sensitivity and promote appetite to push the body toward metabolic homeostasis during the early stages of starvation. It has also been proposed that accumulation of MAT may be a survival mechanism. In this scenario, MAT serves as an energy reserve that can be leveraged during the final stages of starvation to provide several additional days of life.[123] It is also possible that MAT accumulates in response to loss of bone mineral, perhaps regulated by signals from the osteocyte.

MAT as an energetically favorable alternative

The above hypotheses speak to the integration of MAT with whole-body metabolism and survival, but neither provides a rationale for why energy is stored in the skeleton instead of at an extra-skeletal site. In order to answer this question we must first consider the composition and function of the bones. The skeleton fulfills two major roles in an adult: structural support and blood cell production. Bone turnover and hematopoiesis are highly resource intensive and require significant amounts of energy. Marrow fat, on the other hand, is relatively inert in its energy requirements. Thus, from the perspective of the local microenvironment, in times of impaired nutrition, where energy reserves are low, it makes sense to decrease the energy allocation for bone turnover and hematopoiesis in favor of MAT formation. This would favor use of dwindling energy reserves for critical life-sustaining processes. In this scenario, storing energy in the bones as MAT during starvation may make sense from both a systemic and a local perspective. On the other hand, it could be

argued that marrow is life sustaining and that MAT increases the potential energy reserves to maintain basal hematopoietic and skeletal function. Regardless of the underlying mechanism, once nutrition is restored rMAT in particular can be depleted in favor of hematopoiesis.[10]

Temperature-dependent regulation of MAT

The formation and distribution of MAT has also been linked to changes in temperature. Though this theory became well established in the 1930s, many fail to realize that it was repeatedly challenged during the latter half of the 19th century. Since studies pertaining to the relationship between temperature and MAT have been recently revived in the world of MAT research, we will briefly review the evidence for this observation.[124] In 1936, Huggins *et al.* published a study in rats showing that looping the intact, attached tail with implantation of the distal tail vertebrae in the peritoneal cavity of an adult rat results in loss of the mature marrow fat after several months in the implanted vertebrae, with no change in those outside the body cavity.[5] In a similar manner, tail vertebrae harvested from rats shortly after birth and implanted into the peritoneum of an adult host resisted MAT accumulation in the regions of the epiphysis and metaphysis, but not the diaphysis.[5] It was concluded that physiologic temperatures inhibit MAT formation and cause conversion of mature yellow marrow to hematopoietically active tissue. Though this temperature hypothesis of MAT formation rapidly gained favor, follow-up studies in 1956, 1966, and 1979 showed only a mild reduction, or no change, in marrow fat of adult rats after similar experiments.[8,24,125,126] In adult humans, the temperature of the bone marrow is 1.6–4.8 °C below normal body temperature.[127] In six adult patients where the temperature was measured at both the iliac crest (red marrow) and tibia (yellow marrow), the temperature was higher at the iliac site by 0.8–1.8 °C in only three of the six patients.[127] In the other three patients, the temperature was lower by 1.1–2 °C. In the tail of mice, the marrow temperature from proximal to distal decreases by 10 °C.[125] However, the marked transition between hematopoietic and fatty marrow in the first two caudal vertebrae exists independently of any temperature variation.[125] A study in dogs undergoing whole-body hyperthermia showed that, compared to core body temperature, the temperature of the marrow in the ilium, humerus, and tibia was only 0.27 °C, 0.40 °C, and 0.95 °C lower, respectively.[128] The above findings prompt the question, Is a <1 °C variation in temperature across the skeleton actually relevant to marrow fat accumulation and maintenance? The results seem to argue against such a notion in the adult, though the possibility that temperature plays a role in MAT formation during the early stages of development remains intriguing.[24]

While the role of temperature in early MAT development cannot be completely ignored, it must be considered as a component of a much more complex system. For example, induction of hematopoietic demand can synergize with increases in temperature to prevent *de novo* development of MAT in a newborn animal or to reduce MAT in an adult rat.[5,24,129] This reinforces the concept that MAT formation is reciprocally related to hematopoietic demand.

Clinical regulation of MAT

Given the clinical correlations between increases in MAT and decreases in bone, it is not surprising that targeting MAT apoptosis has been proposed as a treatment for osteoporosis.[130] However, without pharmacologic intervention, it is very difficult to get rid of marrow fat (especially cMAT). For example, 24 healthy women 25–40 years of age underwent bed rest for 60 days.[131] During this 60-day period, the fat fraction in the bone marrow as measured by MRI increased by 2.5%. The authors estimate that this increase is 25 times higher than the expected change over 60 days in age-matched ambulatory controls. The increase in MAT persisted 1 year later regardless of exercise and nutritional interventions.[131] In addition, it is well known that the level of MAT increases robustly after irradiation of the skeleton due to local changes in the bone microenvironment.[132] The initial response of bone marrow to irradiation is congestion and edema. The rate of esterification of C^{14}-labeled fatty acids becomes elevated as early as 24 hours after irradiation in rats.[132] In mice, it takes 10–14 days for maximal formation of histologically identifiable MAT after irradiation and bone marrow reconstitution (unpublished observation). In humans, at doses of radiation higher than 50 Gy, it takes at least 6 months for morphological increases in MAT to become evident on MRI.[133] At doses of less than 50 Gy, 1 year or more is

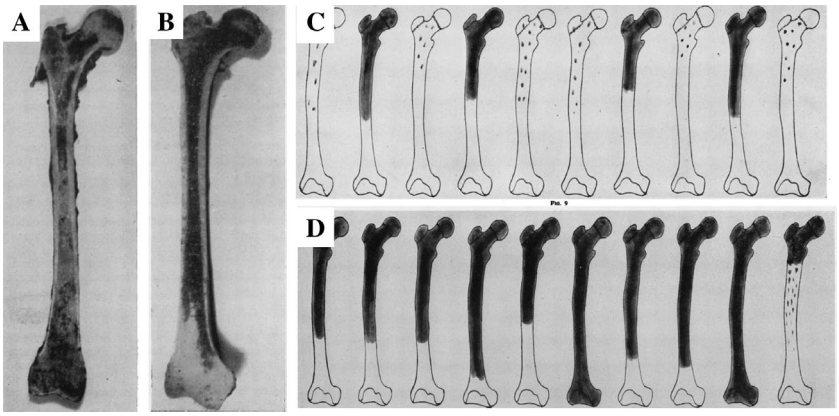

Figure 7. Reconversion of yellow to red marrow in hypertensive heart failure. (A) Control femur from a 61-year-old male. (B) Right femur from a 63-year-old female with hypertensive heart failure. (C) Diagram of marrow content in 10 control female femurs at autopsy (white = yellow marrow, black = red marrow). (D) Diagram of marrow content of 10 femurs from female patients with hypertensive heart failure at autopsy.[136]

needed before injury to the hematopoietic tissue becomes apparent.[133] The difference in timing is striking, but the recovery rate in humans is even more astonishing. Casamassima *et al.* analyzed vertebral MAT content by MRI of 35 patients that underwent radiation therapy.[133] In 22 of the 35 patients, persistent depletion of hematopoietic tissue and accumulation of MAT was observed at time points ranging from 6 months to 15 years after irradiation. Total recovery of the vertebral marrow to its normal ratio of hematopoiesis with loss of MAT was only noted in 11 of 35 patients. This occurred at time points of 7–9 years postirradiation for doses of 1.25 Gy and 10–23 years for doses of >20 Gy.[133] These studies demonstrate the amazing persistence of MAT accumulation in humans. In the setting of irradiation, to limit MAT accumulation it may make sense to target inhibition of MAT formation rather than induction of MAT apoptosis. This is supported by work in rodents. Inhibition of MAT accumulation in transgenic mice undergoing radiation and bone marrow reconstitution increases hematopoietic populations and bone formation postirradiation compared with MAT-rich wild-type controls.[134] In patients who already have high levels of MAT, MAT reduction might be beneficial to bone formation. Mechanical ablation of the marrow (including the MAT) induces a robust healing response, including large amounts of trabecular bone formation. However, this is transient, and newly formed bone returns to baseline within three weeks.[135] This suggests the need to develop strategies for bone maintenance after MAT or marrow removal to have a lasting positive impact.

In human patients, reconversion of normal MAT to hematopoietic marrow generally occurs only in the background of systemic pathology. Shillingford *et al.* reviewed femoral MAT content of 200 patients at autopsy who presented with comorbidities such as hypertension, mitral stenosis, emphysema, aortic valve disease, and cardiac failure.[136] They observed that men have more MAT than women (also true in the C3H mouse strain, unpublished observation) and that red marrow reconversion was specifically associated with hypertension in the presence of heart failure (Fig. 7). Hypertension only (without heart failure) was not sufficient to alter the pattern of red and yellow marrow. Mitral stenosis, aortic valve disease, and emphysema with cardiac failure increased red marrow conversion to a lesser extent.[136] Marathon runners also have higher percentages of red marrow at the distal femur (43%) compared with control individuals (3%) and those with symptomatic knee problems (15%).[137] This may be related to sports anemia, with low red blood cell counts, low hematocrit and/or low hemoglobin levels in otherwise healthy athletes. Induction of anemia in dogs by weekly bleeding resulted in a variable conversion of MAT to red marrow (from 0% to 100%).[138] Anemia-induced lipemia in rabbits is more consistent and, in one study, was prevented by transaction of the spinal cord at the 4th thoracic vertebrae.[139] In light of

this finding, one potential common theme between anemia, cardiac failure, and calorie restriction is the regulation of sympathetic tone. Induction of the sympathetic nervous system may be sufficient to induce lipolysis and/or apoptosis in marrow adipocytes. This is supported by studies in which leptin administered to the brain causes rapid loss of MAT in rats.[140] This is presumed to be due to induction of sympathetic outflow by leptin, though this possibility has not been directly tested. Future studies will be needed to define the ability of the sympathetic nervous system to regulate MAT formation, lipolysis, and apoptosis. Unfortunately, induction of sympathetic tone is also known to increase bone loss;[70] thus, the effects on MAT would need to be separated from the effects on the bone to have a positive clinical impact.

Finally, we have to consider the possibility that MAT is an essential component of the bone marrow microenvironment and that removal might actually be detrimental to skeletal and systemic health. One benefit of MAT is that, though it is difficult to remove, rMAT in particular has the capacity for plasticity when hematopoietic demand is increased. Indeed, rMAT is preferentially depleted in response to phenylhydrazine-induced hemolysis, while cMAT is maintained.[10] In addition, if hematopoietic activity were retained throughout the entire skeleton at the expense of MAT, the energy demand for hematopoiesis would be incredibly costly. MAT, in this way, helps to balance skeletal-energy utilization and blood cell formation.

Finally, we are just beginning to understand the potential of MAT to secrete insulin-sensitizing adipokines in states of profound starvation or lipodystrophy.

Prospects and conclusions

The precise spatial and temporal conservation of MAT development implies that marrow fat formation is a defined developmental event. This suggests that physiologic MAT is an organ that serves an important function and, like other organs, can undergo pathologic changes. We have discussed how pathologies such as osteoporosis may be related not only to the amount of MAT but also perhaps to the proportion of two different types of MAT (rMAT and cMAT). We have also determined, through examining the lipodystrophies, that loss of MAT can contribute to altered skeletal development, cyst formation, and pathologic fracture. In addition, MAT may be a central regulator of skeletal energy allocation between bone turnover and hematopoiesis; and MAT may contribute to systemic metabolism in times of starvation through secretion of adipokines that regulate insulin sensitivity. Thus, it seems that having just the right amount of MAT is essential to skeletal health. Newer animal models and clinical and metabolic studies are likely to shed further light on the origin of marrow adipocytes and their function.

Acknowledgments

This work was supported by supported by grants from the National Institutes of Health (R24 DK092759).

Conflicts of interest

The authors declare no conflicts of interest.

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1. The metabolic and skeletal phenotype of patients with congenital generalized lipodystrophy. Ninety-three patients with congenital generalized lipodystrophy in which some type of skeletal phenotyping was performed are listed with the associated skeletal and clinical findings.

Table S2. Correlation between leptin and bone mineral density in humans. A summary of published studies in which levels of serum leptin were correlated with bone mineral density in humans.

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